CONTRIBUTIONS TO THE BIOLOGY OF RHIZOBIA. IV: TWO COAST RHIZOBIA OF VANCOUVER ISLAND, B. C.[1]

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(WITH THREE FIGURES)

LEGUMINOUS plants are comparatively rare on Vancouver Island. Two species, beach vetch (*Lathyrus maritimus* Bigel.) and beach clover (*Trifolium heterodon* Gray), were more carefully examined with regard to root nodule formation and proved rather interesting. Of these two plants, the vetch is by far the more common and more widely distributed. It is creeping, climbing, and spreading in habit, thus being endowed with certain advantages in the struggle for existence. It climbs upon and spreads over the smaller and less fortunate herbs, thus gaining access to the desirable air and sunlight. The beach clover, in common with the majority of clovers, was originally adapted to the sunlit open ground, but the tree vegetation of the island has compelled it to occupy an extreme shore position. In the struggle for existence it has evolved into a hardy persistent plant, clinging tenaciously to the scant soil in the crevices of rocky shore slopes, approaching the high tide mark. During the often prolonged heavy inland winds of the winter months, these plants are thoroughly drenched by the salt waves and salt spray without suffering any inconvenience whatever. In appearance beach clover is not unlike our familiar white clover (*T. repens*); the plants however are larger, and the flowers are larger and more showy.

The roots of both plants were well supplied with rhizobia-bearing nodules. Sections were made of these and examined microscopically. In the case of beach clover the rhizobia presented the general morphological characteristics of those found in the nodules of red and white clover (*fig. 1*). That is, they were of the very characteristic Indian club form, with very distinct bodies described by some as granules

[1] The work here recorded was done at the Minnesota Seaside Station, session of 1904.

of amylodextrin or degenerate proteids, and which I have elsewhere designated as sporoids. The etiology and function of these bodies still remains to be determined. Some of the rhizobia showed distinct traces of forking (Y-forms), but the majority were of the Indian club form, derived from Y-forms, while a few were of uniform width, evidently derived from simple unbranched rod forms.

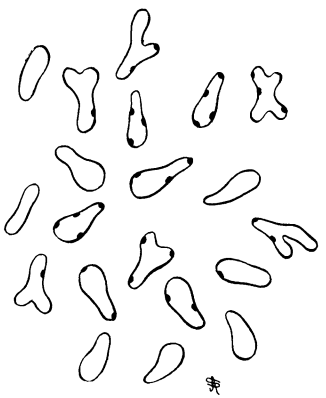

FIG. 1.—Rhizobia from the root nodules of beach clover (*Trifolium heterodon* Gray), showing extreme form variation of *R. mutabile*, due to hyper-nutrition; same organisms with the so-called sporoids.

The rhizobia of beach vetch were rather remarkable for their branching (*fig. 2*). They present the general morphological characteristics of the rhizobia of sweet clover, bur clover, and other vetches. The branching, however, is more pronounced than in any other form of this type hitherto examined. The branching is dichotomous and may be either unipolar or bipolar. The highly refractive sporoids are not present, nor have they ever been observed in rhizobia of this type.

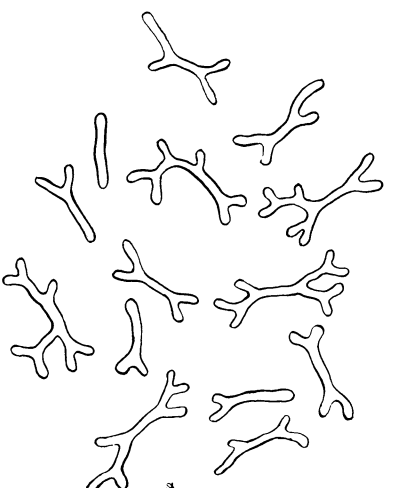

FIG. 2.—Rhizobia from the root nodules of beach vetch (*Lathyrus maritimus* Bigel.), representing the extreme branching form of *R. mutabile*, due to hyper-growth.

It is highly probable that these two rhizobia (of beach vetch and beach clover) represent two extreme natural form types of *Rhizobium mutabile* (*R. leguminosarum* Frank). Assuming that the rod forms and simple Y-forms are the original normal types, we have in the rhizobia of beach clover (and in other clovers) the extreme form deviation, apparently due to hyper-nutrition; and in the rhizobia of

beach vetch the extreme branching form, due to hyper-growth. This supposition is strengthened by the study of rhizobia in artificial culture media. Grown in the same culture media the rhizobia of sweet clover and red clover are morphologically identical. Whether they are physiologically identical has not been determined, although this is also probable according to the inoculation experiments of NOBBE, HILTNER, and HOTTES, of Germany, which are supported by the research of MOORE of the Department of Agriculture. It is quite apparent that these variations in form are due to the food-supply or nutritional changes, variations in the supply of oxygen and moisture, variations in chemical reaction, temperature, light, and other ecological factors. It is possible by means of special culture media to augment very decidedly the branching in the rhizobia of sweet clover and to induce other morphological variations, as has frequently come under my observation. It should be noted that the so-called sporoids do not appear in the rhizobia in artificial culture media. This is of considerable importance, as it was once believed that these bodies were of sufficient significance to be of specific rank. It would appear from these observations that they are more likely by-products stored within the cell, having perhaps food value, derived from the host plant. If this supposition is correct, the theory that they are etiologically sporoidal in nature is untenable.

It seems very probable, and wholly within the range of the possible, that the two extreme form types here described are phylogenetically derived from an original form type similar to, but not necessarily identical with, the form found in *Cassia Chamaecrista*, *Robinia pseudacacia*, *Trigonella foenum graecum*, and *Amphicarpaea comosa*, which were formerly described as distinct species. From this it does not follow, of course, that these form types are of necessity variations of one and the same species, although the evidence thus far deduced points in that direction.

The presence of the *Infectionsfäden* was noted (*fig. 3*), and nothing new regarding their significance was discovered, only I wish to state, by way of readjustment of what was stated in previous papers, that in all probability these threads are merely a phenomenon dependent upon the infection of the root cells by the rhizobium. The action of the motile forms in the apical area of the root nodule (formerly

described as a distinct species, *R. Frankii*) causes the appearance of the threads in a manner already explained.

The question of Rhizobia species is not yet settled, and cannot be settled until our information regarding their biology is much more complete. The fact that extensive research work has already been done by a large number of investigators without coming to any conclusion regarding species should serve as a very suggestive lesson

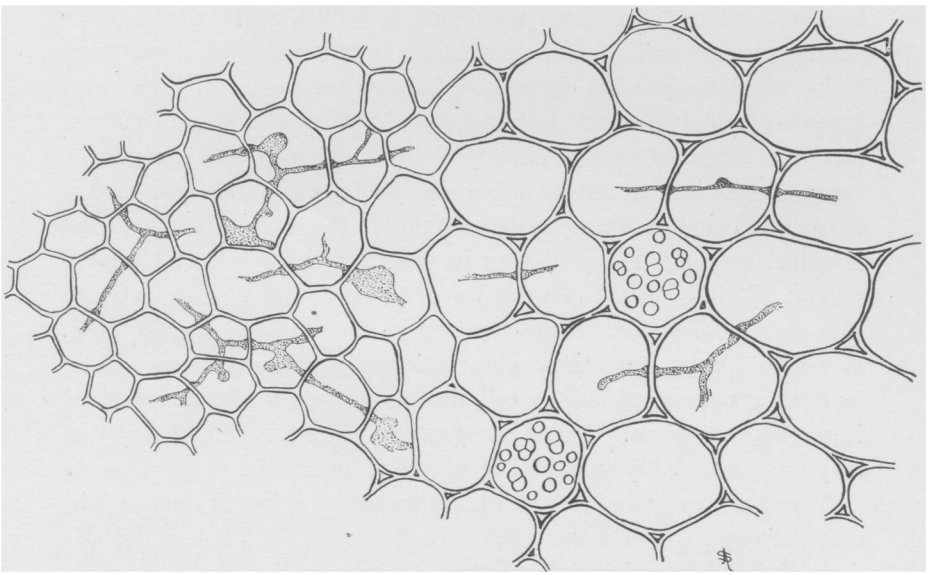

FIG. 3.—Portion of longitudinal section of root nodule of beach vetch (*Lathyrus maritimus* Bigel.) showing *Infectionsfäden*, more abundant in the apical area; rhizobia are not shown; two starch-bearing cells.

to species hunters. In the case of *R. mutabile*, the question is now arising as to whether it is a microbe (Schizomycete) or a hyphal fungus, a question which we hope to discuss more fully in some future paper. All investigators are agreed that *R. mutabile* is an organism showing extreme polymorphism. It would appear to be an organism wonderfully adapted to test the De Vriesian theory of mutation as it applies to low organisms. At this time the only statement ventured is that apparently constant natural variations in *R. mutabile*, as above indicated, at once become transformed into "variable or unstable vari-

ability" in artificial culture media. It would appear that the newer conception of species as based upon the facts of ecology; study of mutation, constant and variable; crossing, artificial and natural, etc., will necessitate a complete change in our present systems of classification.

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